American Journal of Hematology 63:79–84 (2000)

Atypical β^S Haplotypes Are Generated by Diverse Genetic Mechanisms

M.A. Zago,[1]* W.A. Silva, Jr.,[1] B. Dalle,[2] S. Gualandro,[3] M.H. Hutz,[4] C. Lapoumeroulie,[2] M.H. Tavella,[1] A.G. Araujo,[1] J.E. Krieger,[3] J. Elion,[2] and R. Krishnamoorthy[2]

[1]Department of Clinical Medicine and Hemocentro, School of Medicine, Ribeirão Preto, Brazil
[2]Laboratoire de Biochimie Génétique, INSERM U458, Paris, France
[3]Department of Clinical Medicine, Faculty of Medicine of the University of S. Paulo, Brazil
[4]Department of Genetics, Federal University of Rio Grande do Sul, Brazil

The majority of the chromosomes with the β^S gene have one of the five common haplotypes, designated as Benin, Bantu, Senegal, Cameroon, and Arab-Indian haplotypes. However, in every large series of sickle cell patients, 5–10% of the chromosomes have less common haplotypes, usually referred to as "atypical" haplotypes. In order to explore the genetic mechanisms that could generate these atypical haplotypes, we extended our analysis to other rarely studied polymorphic markers of the β^S-gene cluster, in a total of 40 chromosomes with uncommon haplotypes from Brazil and Cameroon. The following polymorphisms were examined: seven restriction site polymorphisms of the $\varepsilon\gamma\delta\beta$-cluster, the pre-$^G\gamma$ framework sequence including the 6-bp deletion/insertion pattern, HS-2 LCR (AT)xR(AT)y and pre-β (AT)xTy repeat motifs, the GC/TT polymorphism at −1105–1106 of $^G\gamma$-globin gene, the C/T polymorphism at −551 of the β-globin gene, and the intragenic β-globin gene framework. Among the Brazilian subjects, the most common atypical structure (7/16) was a Bantu 3′-subhaplotype associated with different 5′-sequences, while in two chromosomes a Benin 3′-subhaplotype was associated with two different 5′-subhaplotypes. A hybrid Benin/Bantu configuration was also observed. In three chromosomes, the atypical haplotype differed from the typical one by the change of a single restriction site. In 2/134 chromosomes identified as having a typical Bantu RFLP-haplotype, a discrepant LCR repeat sequence was observed, probably owing to a crossover 5′ to the ε-gene. Among 80 β^S chromosomes from Cameroon, 22 were associated with an atypical haplotype. The most common structure was represented by a Benin haplotype (from the LCR to the β-gene) with a non-Benin segment 3′ to the β-globin gene. In two cases a Bantu LCR was associated with a Benin haplotype and a non-Benin segment 3′ to the β-globin gene. In three other cases, a more complex structure was observed that can be considered as a hybrid of Benin, Bantu, Senegal, or other chromosomes was observed. These data suggest that the atypical β^S haplotypes are not uncommon in America and in Africa. These haplotypes are probably generated by a variety of genetic mechanisms including (a) isolated nucleotide changes in one of the polymorphic restriction sites, (b) simple and double crossovers between two typical β^S haplotypes or much more frequently between a typical β^S haplotype and a different β^A-associated haplotype that was present in the population, and (c) gene conversions. Am. J. Hematol. 63:79–84, 2000.

Key words: atypical haplotypes; sickle cell anemia; polymorphism

INTRODUCTION

The differing genetic background of the human β-globin gene cluster on chromosome 11 has usually been recognized by a set of polymorphic restriction enzyme profiles of this DNA region which constitute the β globin gene cluster haplotype. The β^S gene responsible for sickle cell anemia has been found to be associated with

Contract grant sponsors: FAPESP; CNPq (Brazil); European Commission; COFECUB; Contract grant number: TS3*CT93-0244 (EC).

*Correspondence to: M.A Zago, Department of Clinical Medicine, School of Medicine of Ribeirão Preto, 14049-900 Ribeirão Preto, Brazil. E-mail: marazago@usp.br

Received for publication 12 December 1998; Accepted 6 October 1999

five different haplotypes, namely, Benin, Bantu, Senegal, Cameroon, and Arab-Indian, named after the regions or ethnic groups in which the designated β^S haplotype was common [1–5]. Such a geographic prevalence of the β^S-gene associated with specific haplotypes has been argued to demonstrate the independent origin of the β^S-mutation in these regions [2,3,5]. This assumption has been rejected by others who favor a unicentric origin of the β^S mutation, that would have had spread to different haplotypes by an yet to be substantiated process [6]. The present day β^S genes in different parts of the American continent and Europe are imported from Africa, and the proportion of the associated haplotypes is believed to represent the haplotype stock originally present in the imported slaves and of the more recent immigrants. In addition to the anthropologic and genetic interest, the polymorphisms linked to the β^S gene may behave as markers to explain the genetic differences in the clinical expression of sickle cell anemia as well as the variations in drug response [3]. The majority of the chromosomes with the β^S gene have one of the five common haplotypes, although in every large series of sickle cell patients there is a minority of chromosomes (5–10%) associated with less common haplotypes, usually referred to as "atypical" haplotypes. Little attention has been paid to these atypical haplotypes. One study refers to them as variations of the Bantu haplotype whereas other reports consider them as the product of recombination of Bantu haplotypes [7–9].

In the present study we performed an extended analysis of the polymorphic markers of the β^S-gene cluster of 40 chromosomes with atypical haplotypes and demonstrated that they can be generated by several genetic mechanisms, involving the Bantu, the Benin, or the Senegal haplotypes.

MATERIALS

This study comprises 16 unrelated sickle cell anemia patients not previously reported. Among 204 Brazilian HbS homozygotes there were 14 patients with atypical β^S-haplotypes as detected by digestion with 7 restriction enzymes. The samples were collected from patients under regular follow-up in university hospitals from three cities in the South and Southeast of Brazil (Ribeirão Preto, São Paulo, and Porto Alegre). All samples collected were included in the study, without further selection. Among 67 patients with typical haplotypes (either Benin or Bantu homozygotes, or compound heterozygotes) the investigation of the HS-2 site of the LCR region (see below) revealed two cases with a Bantu haplotype and a discrepant LCR structure. In addition, we present new extended data on 22 chromosomes with atypical haplotypes observed among 40 out-patients followed at the Blood Transfusion Center of the Central Hospital in Yaoundé, the capital city of Cameroon and previously reported [5].

METHODS

All patients were HbS homozygotes as determined by hemoglobin electrophoresis and by *Mst*II digestion of a β-globin gene segment obtained by PCR. The β^S-haplotypes (based on seven restriction sites) was determined by PCR followed by restriction enzyme digestion as previously described [10]. PCR-based methods have been used to explore further the following 6 regions of the β-globin cluster as follows: (1) The (AT)xTy motif was amplified and directly sequenced according to the procedure described by Trabuchet et al [11]. In heterozygotes, the PCR product was cloned by the TA Cloning kit (Invitrogen, Carlsbad, CA), and the clones were sequenced with the T7 Sequencing kit (Pharmacia). Within the same segment, the C/T polymorphism at −551 of the β-globin gene (*Rsa*I site) was determined. (2) The 6-bp deletion at −400/395 nt at 5′ to the $^G\gamma$-globin gene was detected by amplification of a 640-bp segment followed by direct observation of the product size and heterodimer formation on electrophoresis [12]. (3) The GC/TT polymorphism at nt −1106/5 at 5′ to the $^G\gamma$-globin gene was determined by amplification of a 158-bp segment and direct sequencing [13]. (4) The pre-$^G\gamma$-gene framework [14] between −1450 and −1225 nt at 5′ to the $^G\gamma$-gene was determined by amplification and direct sequencing. (5) To explore the (AT)xN12(AT)y repeat sequence configuration within the HS-2 region of the β-LCR, a 324-bp fragment was amplified [15]. The PCR product was cloned and sequenced as described above. Alternatively, it was also analyzed by amplification and digestion of the 324-bp fragment with *Xba*I and *Hin*fI followed by electrophoresis of the product on 7% polyacrylamide for 4 hr at 180 V. In addition to the 47-, 77-, and 87-bp constant fragments, a fragment of variable size between 104 and 120 bp is observed, depending on the size of the (AT)xN12(AT)y repeat. For the Bantu and the Benin typical motifs, respectively, a 110- or a 104-bp fragment was obtained (Fig. 1). (6) The β-globin gene frameworks were defined by DGGE of a 474-bp fragment amplified by primer pair G as described by Ghanem et al. [16].

For the patients from Cameroon, the pre-$^G\gamma$ haplotypes were not determined, and the restriction enzyme haplotyping was extended to *Hin*fI, *Hin*dIII, *Bam*HI, and *Hpa*I 3′ to the β-globin gene. *Hin*fI and *Hpa*I sites were determined by Southern blotting using appropriate probes.

RESULTS

The results obtained for the 16 Brazilian patients are shown in Table I. Wherever relevant, for haplotype con-

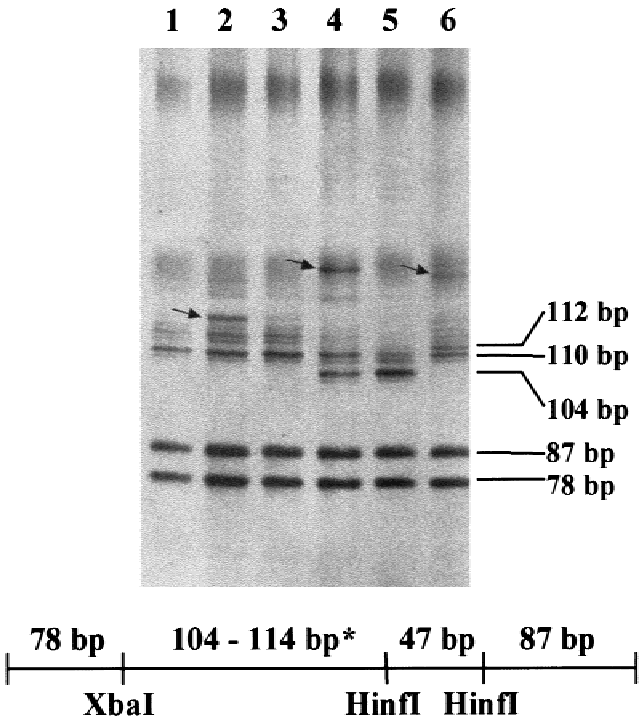

Fig. 1. Identification of different repeat structures in the HS-2 region of the LCR. Digestion of the 324-pb segment (indicated at the bottom) with *Xba*I and *Hin*fI produces three constant-size fragments (87, 78, and 47 bp) and a variable size fragment (*) of 104 to 114 bp, depending on the number of repeats. In comparison with the constant-size bands, the variable size bands are less well defined and accompanied by secondary bands probably owing to structure formation. Heterodimer bands are also observed in heterozygotes (arrows in lanes 2, 4, and 6). Lane 1, Bantu/Bantu; lane 2, Bantu/Senegal; lane 3, Senegal/Senegal; lane 4, Bantu/Benin; lane 5, Benin/Benin; lane 6, Benin/Atypical.

struction, it was assumed that the genotype constitution was a combination of one common and one atypical haplotype, rather than the presence of two atypical haplotypes. Accordingly, a combination of a common and an atypical haplotype was observed in 12/14 cases (four Benin/atypical and eight Bantu/atypical), whereas a combination of two atypical haplotypes was the only possibility in two patients (cases 5 and 6), making up a total of 16 chromosomes with an atypical β^S-cluster haplotype, i.e., 3.92% of the 408 β^S chromosomes.

Table II summarizes the structure of these 18 atypical haplotypes. The most common structure, found in 7/16 chromosomes (cases 1–5 and the two chromosomes of case 6), was a Bantu 3′-subhaplotype (including the *Rsa*I$^+$ site, an (AT)6T9 repeat sequence, and a β-globin gene framework 1) associated with different 5′-sequences. In three chromosomes (cases 4, 5, and 6) the LCR structure was of the Bantu type, and in two of them this was associated with a *Hinc*II$^+$ site at 3′ to the ψβ-gene. In two chromosomes (cases 5 and 7) a Benin 3′-subhaplotype (including a *Hinc*II$^+$ site at 3′ to the ψβ-gene, an *Rsa*I$^-$ site, an (AT)8T4 repeat sequence, and a β-globin gene framework 2) was found in each case associated with a different 5′-subhaplotype. A clear example of a Benin/Bantu hybrid was observed in a patient (case 8) in whom a 5′-region was similar to the Benin type (including a (AT)8N12GT(AT)7 at the HS2-LCR, a pre-$^G\gamma$ framework 1 and a *Hin*dIII$^-$ site at $^G\gamma$) but the 3′ region was Benin/Bantu, from the *Hinc*II$^{+/-}$ site at 3′ to the ψβ-gene, an *Rsa*I$^{+/-}$ site, a (AT)6T9/(AT)8T4 repeat and β-globin gene frameworks 1 and 2.

In two cases (cases 9 and 10), the complete haplotype was atypical, with a positive *Hin*fI site at 5′ of the β-globin gene, a positive *Rsa*I site, a (AT)7T7 repeat sequence and framework 1. For one chromosome (case 11) the 5′ structure was Benin-like, including an (AT)8N12GT(AT)7 repeat sequence at the HS2-LCR and a pre-$^G\gamma$ framework 3, while the 3′ subhaplotype was completely atypical, with an (AT)9T4 repeat sequence and a CT mutation at nt −521, and β-globin sequence framework 1.

In three chromosomes the atypical haplotype differed from the typical one exclusively by a single restriction enzyme site (cases 12, 13, and 14). In two of them, a complete Bantu-type β-globin gene cluster, from the HS2-LCR to the β-globin sequence framework, had a *Hin*dIII-negative site at $^G\gamma$ (instead of positive) in one case, and a *Hinc*II-positive site at 3′ to the ψβ-gene (instead of negative) in the other. In the third case, a complete Benin-type extended haplotype was associated with the absence of the *Hinc*II site 3′ to the ψβ-gene (instead of its usual presence on the typical chromosome).

Among 67 patients with typical RFLP β^S haplotypes (33 Bantu homozygotes, 5 Benin homozygotes, and 29 Bantu and Benin compound heterozygotes), the electrophoretic analysis of the amplified and digested HS-2 region of the LCR agreed with the haplotype in 65 cases. In two Bantu homozygotes, however, electrophoresis showed a discrepant HS2-LCR fragment size; upon nucleotide sequencing of this segment, it was found to be heterozygote in both cases for the (AT)xN12(AT)y repeat [(AT)8N12(AT)11/(AT)10N12(AT)11], while all the remaining markers were Bantu type (cases 15 and 16).

Finally, 22 atypical chromosomes were observed among the 80 chromosomes from Cameroon and were previously reported as Benin/Bantu hybrids. However, refined haplotyping carried out in this study demonstrated that they probably represent recombinations either between segments of chromosomes with typical haplotypes, or with other chromosomes. The most common structure was represented by a Benin haplotype (from LCR to β-globin gene) with a non-Benin segment 3′ to the β-globin gene, with the presence of a *Hpa*I site instead of the its absence characteristic of the typical Benin haplotype. In two cases a Bantu HS2-LCR configuration

TABLE I. Extended β-Cluster Haplotypes of 16 HbS Homozygotes With Atypical RFLP Haplotypes, or With a Discrepant LCR in Relation to RFLP Haplotypes

Case	LCR (HS-2)[a] (AT)xR(AT)y	ε-Gene *Hin*cII ε	$^{G}\gamma$-Gene Pre-$^{G}\gamma$ haplotype	$^{G}\gamma$-Gene −1106/1105 $^{G}\gamma$	$^{G}\gamma$-Gene −403/390 $^{G}\gamma^{b}$	γδβ-Cluster[c] 6-Site haplotype	β-Gene −551 T/C (*Rsa*I)	β-Gene −530 (AT)xTy	Framework
1	8N′7/8N10	−/−	1/3	GC/GC	ND/ND	−−−−+−/−+−−+−	T/C	8.4/6.9	2/1
2	8N′7/10N11	−/+	1/1	GC/GC	ND/ND	−−−−+−/−−−−−−	T/C	8.4/6.9	2/1
3	8N11/8N14	−/−	3/1	TT/GC	D/ND	−+−−−−/−−−−−−	C/C	6.9/6.9	1/1
4	8N11/8N11	−/+	3/1	TT/GC	D/ND	−+−−−−/−−−−−−	C/C	6.9/6.9	1/1
5	8N11/8N10	−/+	1/1	GC/GC	ND/ND	−−−−+−/−−−−+−	C/T	6.9/8.4	1/2
6	8N11/9N11	−/+	1/1	GC/GC	ND/ND	−−−−+−/−−−−−+	C/C	6.9/6.9	1/1
7	8N11/9N14	−/−	3/3	TT/GC	D/ND	−+−−−−/−+−−+−	C/T	8.4/6.9	1/2
8	8N′7/8N′7	−/−	1/1	GC/GC	ND/ND	−−−−+−/−−−−−−	T/C	8.4/6.9	2/1
9	8N11/9N12	−/+	3/1	TT/GC	D/ND	−+−−−−/−−−−−+	C/C	6.9/7.7	1/1
10	8N11/8N10	−/−	3/1	TT/GC	D/ND	−+−−−−/−−−−++	C/C	6.9/7.7	1/1
11	8N11/8N′7	−/−	3/3	TT/GC	D/ND	−+−−−−/−+−−++	C/T	6.9/9.4[d]	1/1
12	8N11/8N11	−/−	3/3	TT/TT	D/D	−+−−−−/−−−−−−	C/C	6.9/6.9	1/1
13	8N11/8N11	−/−	3/3	TT/TT	D/D	−+−−−−/−+−−+−	C/C	6.9/6.9	1/1
14	8N′7/8N′7	−/−	1/1	GC/GC	ND/ND	−−−−+−/−−−−−−	T/T	8.4/8.4	2/2
15	8N11/10N11	−/−	3/3	TT/TT	D/D	−+−−−−/−+−−−−	C/C	6.9/6.9	1/1
16	8N11/10N11	−/−	3/3	TT/TT	D/D	−+−−−−/−+−−−−	C/C	6.9/6.9	1/1

[a]R: N = ACA CAT ATA CGT and N′ = ACA CAT ATA CGT GT.
[b]6-bp deletion or non-deletion.
[c]*Xmn*I at 5′ to $^{G}\gamma$, *Hin*dIII at $^{G}\gamma$, *Hin*dIII at $^{A}\gamma$, *Hin*cII at ψβ, *Hin*cII at 3′ to ψβ, *Hin*fI at 5′ to β.
[d]−521 C → T.

was associated with a Benin haplotype and a non-Benin segment at the 3′ part of the β-globin gene. In three other cases, complex structures, presumably hybrids of Benin, Bantu, Senegal or other chromosomes were observed. Figure 2 summarizes the findings of the 40 uncommon haplotypes.

DISCUSSION

Our results demonstrate diverse genetic mechanisms generate the atypical haplotypes associated with the sickle cell gene, i.e., the haplotypes that differ from the five most common haplotypes observed worldwide. The data strongly support the idea that all of these different structures are generated by recombinations or punctual substitutions or nonreciprocal sequence transfer (conversion) in the pre-existing common haplotypes instead of recurrent de novo β^{S} mutations.

The 40 uncommon haplotypes observed in the present study can be grouped in at least 14 different patterns. In three cases from Brazil, the atypical haplotypes originated from a common haplotype by the gain or the loss of a single restriction site as a result of point mutations. Much more common were the cases in which the atypical haplotype was a hybrid between the 3′ segment containing the β^{S} gene very likely from a typical haplotype and the 5′ segment derived from another chromosome. In a single case, the hybrid was clearly formed by the 5′ segment of a Bantu chromosome and the 3′ segment of a Benin chromosome, either a Benin β^{S} chromosome or a Benin-like β^{A}; in other cases, the 5′ region did not belong to one of the common β^{S}-associated haplotypes, and was probably derived from β^{A} chromosomes. In addition, in 2/134 chromosomes with typical Bantu or Benin haplotypes, extended haplotyping demonstrated a discrepant HS2-LCR sequence configuration, indicating again occurrence of recombination between a Bantu haplotype and another chromosome. Thus, from the 12 atypical chromosomes possibly originated by recombination, the most frequent recombination site is the previously proposed hotspot 5′ of the δ-globin gene (10 chromosomes), followed by a potential recombination

TABLE II. Structure of the β^{S}-Globin Gene Clusters Observed in 16 Chromosomes of 14 Patients with Atypical 7-Restriction Site Haplotypes, and Two Patients With Typical Haplotypes and a Discrepant HS-2 LCR Repeat Motif

LCR	5′ Segment	3′ Segment	No.	Case number(s)
Variable	Variable	Bantu	7[a]	1–5, 6[b]
Variable	Variable	Benin	2	5,7
Benin	Benin	Bantu	1	8
Atypical	Atypical	Atypical	3	9,10
Benin	Benin	Atypical	1	11
Bantu	Bantu[c]	Bantu	1	12
Bantu	Bantu	Bantu[c]	1	13
Benin	Benin	Benin[c]	1	14
Subtotal			16	
Atypical	Bantu	Bantu	2	15,16
Total			18	

[a]Two cases are similar to the Bantu/Benin/Bantu structure previously reported [8].
[b]Two atypical chromosomes.
[c]With a point mutation that creates or abolishes a restriction site.

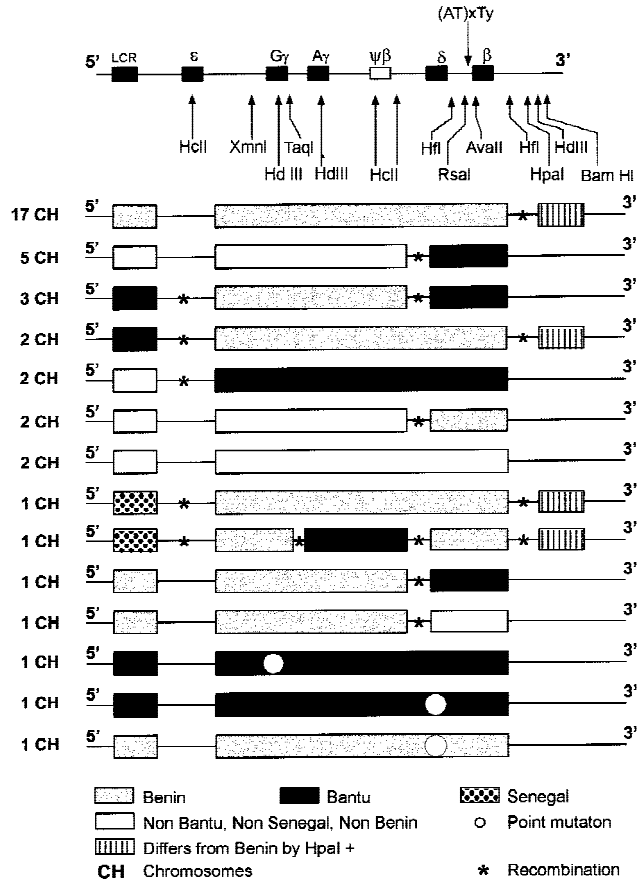

Fig. 2. The structure of 40 atypical haplotypes associated with chromosomes with the β^S gene. In 24 cases, the markers near and within the β^S gene (3′ region) were identical to those observed in the Benin haplotype, but differed from it either in the 5′ region or in the segment at 3′ of the β^S gene. In 13 cases, the markers near and within the β^S gene are identical to those of the Bantu haplotype, while the markers at the 5′ region are different. In three cases the haplotype was typically Benin or Bantu, except for a single restriction site.

between the ε gene and the HS2-LCR region (two chromosomes). Finally, in two cases the β^S mutation was part of a β-globin gene cluster totally unrelated to any other common β^S haplotypes. These cases could represent de novo mutations, or most likely result from recombinations 3′ to the (AT)xTy repeat or from gene conversion. It is interesting to note that the contribution of the two microsatellite-type simple sequence repeats to further diversity of the classical six-site RFLP-haplotypes is much greater than other additional polymorphic positions explored in this study. Indeed these sequences are known to contribute to allelic variability through their intrinsic plasticity [17].

The analysis of 22 atypical chromosomes from Cameroon demonstrates that these mechanisms are also not uncommon within Africa.

Most of the cases from Brazil represent mutations or recombinations involving the Bantu β^S-gene; in two previous studies [7,8], the structure of the atypical haplotypes revealed recombinations only of the Bantu haplotype. This is probably due to the fact that all those studies were carried out in regions where the Bantu haplotype predominates: Angola, Mozambique, and Brazil [18,19]. As we show here, the Benin β^S gene can also be target of the same mechanisms. Similar mechanisms of point mutations and recombinations involving Bantu, Benin, and Senegal haplotypes could be inferred as the source of some of the atypical haplotypes in another study [20].

Only two of the 14 haplotypes observed among these cases were recurrent. The most frequent of them was a haplotype that differs from the typical Benin haplotype by the presence of a positive *Hpa*I site 3′ to the β^S gene, instead of its characteristic absence in Benin β^S chromosomes. This could be caused either by a point mutation, creating a restriction enzyme site, or by recombination at the 3′ part of the β^S gene. A high frequency of this haplotype was observed among the Cameroon patients (17/80 chromosomes, or 21.2%). Since this site is not routinely tested in most studies, it is not possible to evaluate how frequently this haplotype occurs in other populations, but it has been observed in 6.7% of β^S chromosomes from Brazil [18]. Thus, it seems that a Benin haplotype with a non-Benin segment at 3′ of the β^S gene has achieved high frequencies among some population groups. Although the pattern of a Bantu 3′ segment associated with a non-Bantu 5′ segment including HS2-LCR was observed six times, the actual haplotypes were different in each case, and each case may represent a new recombination event. By contrast, the pattern of a Benin 5′ segment associated with a Bantu HS2-LCR and Bantu 3′ segment was observed three times and found to be identical the Bantu/Benin/Bantu mosaic structure described previously [8]. It would represent another example of an uncommon β^S haplotype that dispersed among different population groups or, although less likely, recurrently generated.

On the other hand, our study design is such that the generated data are not sufficient to deal with the issue of the uni- or multicentric origin of the sickle cell gene(s), especially when data is lacking concerning many other African populations with regard to their β-globin cluster haplotypes associated with β^A or β^S genes.

In conclusion, we demonstrate that the atypical haplotypes observed in association with sickle cell gene are likely to be generated by diverse genetic mechanisms, involving either the Bantu or the Benin β^S-chromosome, including (a) point mutations in typical haplotypes, (b) simple and double recombinations between a β^S chromosome either with a common β^S haplotype or a less common haplotype associated with a β^A chromosome present in given populations, and (c) gene conversion. The overall picture of the β-globin gene cluster derived

from our study is that the cluster is highly dynamic. Furthermore, the data warrants caution when conclusions are drawn assuming that the β^S-associated haplotypes are highly stable, especially in long-range population studies. The studies of phenotype-genotype relationship using the incomplete classical haplotype data need to be revisited as some of the additional polymorphic segments herein studied are in functionally relevant regulatory regions [21].

ACKNOWLEDGMENT

The authors are indebted to Ms Iara K. Yokomizu for her technical assistance.

REFERENCES

1. Antonarakis SE, Boehm CD, Serjeant GR, Theisen CE, Dover GJ, Kazazian HH. Origin of the β^S-globin gene in Blacks: The contribution of recurrent mutation or gene conversion or both. Proc Natl Acad Sci USA 1984;81:853–856.
2. Pagnier J, Mears JG, Dunda-Belkhodja O, Schaefer-Rego KE, Beldjord C, Nagel RL, Labie D. Evidence for the multicentric origin of the sickle cell hemoglobin gene in Africa. Proc Natl Acad Sci USA 1984; 81:1771–1773.
3. Nagel RL, Fabry ME, Pagnier J, Zohoun I, Wajcman H, Baudin V, Labie D. Hematologically and genetically distinct forms of sickle cell anemia in Africa. The Senegal type and the Benin type. N Engl J Med 1985;312:880–884.
4. Kulozik AE, Wainscoat JS, Serjeant GR, Kar BC, Al-Awamy B, Essan GJF, Falusi AG, Haque SK, Hilali AM, Kate S, Ranasinghe WAEP, Weatherall DJ. Geographic survey of β^S-globin gene haplotypes: evidence for the independent Asian origin of the sickle-cell mutation. Am J Hum Genet 1986;39:239–244.
5. Lapouméroulie C, Dunda O, Ducrocq R, Trabuchet G, Mony-Lobé M, Bodo JM, Carnevale P, Labie D, Elion J, Krishnamoorthy R. A novel sickel cell mutation of yet another origin in Africa: The Cameroon type. Hum Genet 1992;89:333–337.
6. Flint J, Harding RM, Clegg JB, Royce AJ. Why are some genetic diseases common? Distinguishing selection from other processes by molecular analysis of globin gene variants. Hum Genet 1993;91:91–117.
7. Srinivas R, Dunda O, Krishnamoorthy R, Fabry M E, Georges A, Labie D, Nagel RL. Atypical haplotypes linked to the β^S gene in Africa are likely to be the product of recombination. Am J Hematol 1988;29:60–62.
8. Gonçalves I, Gonçalves J, Périchon B, Osório-Almeida L, Krishnamoorthy R, Lavinha J. A novel mosaic Bantu/Benin/Bantu β^S haplotype found in several African populations. Hum Genet 1994;94: 101–103.
9. Öner C, Dimovski AJ, Altay Ç, Gurgey A, Gu YC, Huisman THJ, Lanclos KD. Sequence variations in the 5′ hypersensitive site-2 of the locus control region of β^S chromosomes are associated with different levels of fetal globin in hemoglobin S homozygotes. Blood 1992;79: 813–819.
10. Sutton M, Bouhassira EE, Nagel RL. Polymerase chain reaction amplification applied to the determination of β-like globin gene cluster haplotypes. Am J Hematol 1989;32:66–69.
11. Trabuchet G, Elion J, Baudot G, Pagnier J, Bouhass R, Nigon VM, Labie D, Krishnamoorthy R. Origin and spread of β-globin gene mutations in Indian, Africa and Mediterranean: Analysis of the 5′ flanking and intragenic sequences of β^S and β^C genes. Hum Biol 1991;63: 241–252.
12. Bouhassira EE, Nagel RL. A 6-bp deletion 5′ to the $^G\gamma$ globin gene in β^S chromosomes bearing the Bantu haplotype. Am J Genet 1990;47: 161–163.
13. Tachdjian G, Benabdennebi M, Guidal C, Sayada C, Lapouméroulie C, Elion J. Analysis of the 5′ flanking sequence of the $^G\gamma$ globin gene by denaturing gradient gel electrophoresis confirms the heterogeneity of the Bantu β^S haplotype. Hum Genet 1992;90:23–26.
14. Pissard S, Beuzard Y. A potential regulatory region for the expression of fetal hemoglobin in sickle cell disease. Blood 1994;84:331–338.
15. Périchon B, Ragusa A, Lapouméroulie C, Romand A, Moi P, Ikuta T, Labie D, Elion J, Krishanamoorthy. Inter-ethnic polymorphism of the β-globin gene locus control region (LCR) in sickle-cell anemia patients. Hum Genet 1993;91:464–468.
16. Ghanem N, Girodon E, Vidaud M, Martin J, Fanen P, Plassa F, Goossens M. A comprehensive scanning method for rapid detection of β-globin gene mutations and polymoprphisms. Hum Mutat 1992;1: 229–239.
17. Jeffreys AJ, Royle NJ, Wilson V, Wong Z. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature 1988;332:278–281.
18. Zago MA, Figueiredo MS, Ogo SH. Bantu β^S cluster haplotype predominates among Brazilian Blacks. Am J Phys Anthropol 1992;88: 295–298.
19. Figueiredo MS, Olympio da Silva MCB, Guerreiro JF, Pante de Souza G, Pires ACR, Zago MA. The heterogeneity of the β^S cluster haplotypes in Brazil. Gene Geography 1994;8:7–12.
20. Lu ZH, Steinberg MH. Fetal hemoglobin in sickle cell anemia: Relation to regulatory sequences cis to the β-globin gene. Multicenter Study of Hydroxyurea. Blood 1996;87:1604–1611.
21. Labie D, Elion J. Sequence polymorphisms of potential functional relevance in the β-globin gene locus. Hemoglobin 1996;20:85–101.